Dear, editor

Thank you again for your interest in this study and for your consideration of publication. The authors have done our best to respond to the comment raised by the reviewers and science editor by a point-by-point manner. Your comments were in **bold**. The revised sentences or words are colored by "Red" in the related manuscript and in the answer. The authors' explanation is underlined.

Sincerely, SJ Lee as a corresponding and communicating author

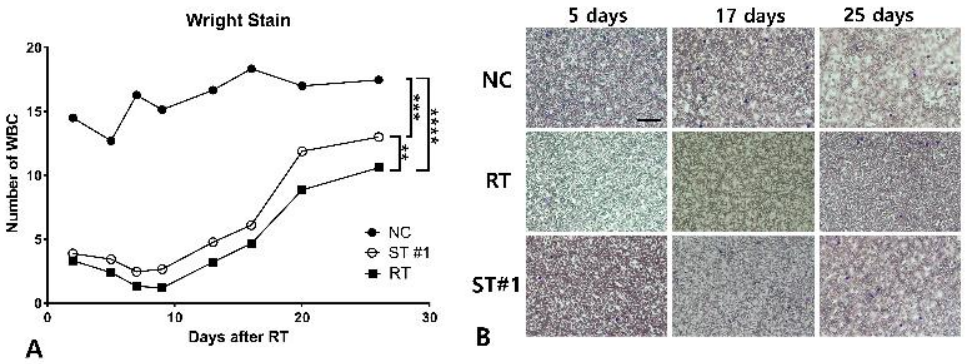
Reviewer #1:

"Optimization of adipose tissue-derived mesenchymal stem cells transplantation for bone marrow repopulation after irradiation" by Won Moon et al. In my opinion, the topic is interesting, since "Bone marrow suppression is one of the most common side effects of radiotherapy" The authors report that single peritoneal administration of adipose-derived mesenchymal stem cells increase the survival rate of mice, if compared with mice transplanted three times. Moreover, it is suggested that such intraperitoneal administration might suppress erythropoiesis and improve myelopoiesis in sub-lethally irradiated mice Methods are satisfactorily carried out and Results are clearly presented.

1. However, it should be better emphasized the clinical relevance of these findings.
   : Thanks for the good point. Regarding clinical relevance, the following phrase is inserted in the introduction section. (line 94)
   "Hematopoietic symptoms, particularly those induced by ARS, develop into severe neutropenia and thrombocytopenia, which eventually lead to bleeding, infection, and death.[3] The same clinical results are obtained when myeloablative therapy is performed in patients with conditions such as leukemia, lymphoma, and myeloma.[4]"

2. Line 382: Alizarin Red S and Alcian Blue staining are likely inverted.
   : The part the reviewer said has been corrected as follows. (line 197)
   "In the adipogenic, chondrogenic, and osteogenic groups, the culture medium was replaced every 2 d and the cells were stained with Oil Red O (Sigma-Aldrich), Alcian Blue (Sigma-Aldrich), and Alizarin Red S (Sigma-Aldrich), respectively, to confirm differentiation"

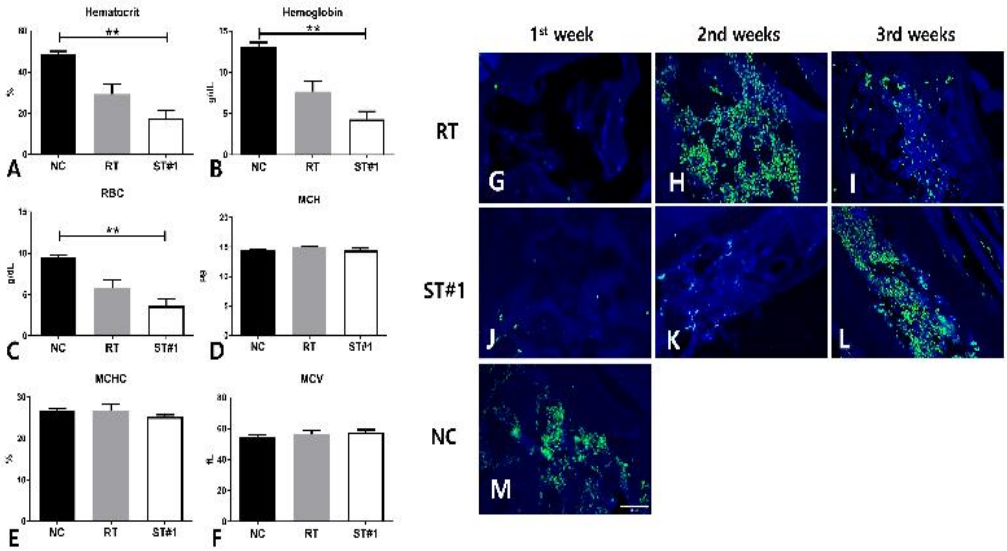
3. Line 754: In the legend to Fig.2, description of panel A and panel B are likely inverted. Check magnification (200x ?).
   : The positions of panel A and B in Fig.2 were exchanged as follows. The magnification was confirmed that the photo was taken at 200x magnification. (line 839)



4. Figure 2: Photomicrographs should be enlarged and a scale bar should be indicated.
   : Yes, the authors have enlarged the Wright stain picture as shown in the picture above and adjusted the resolution again. A scale bar (20 μm) was inserted. (line 843)

5. Line 767: Check magnification (400x?) in the legend to Fig.3.
   : The authors have checked the magnification again. This photo was taken at 400x. (line846)

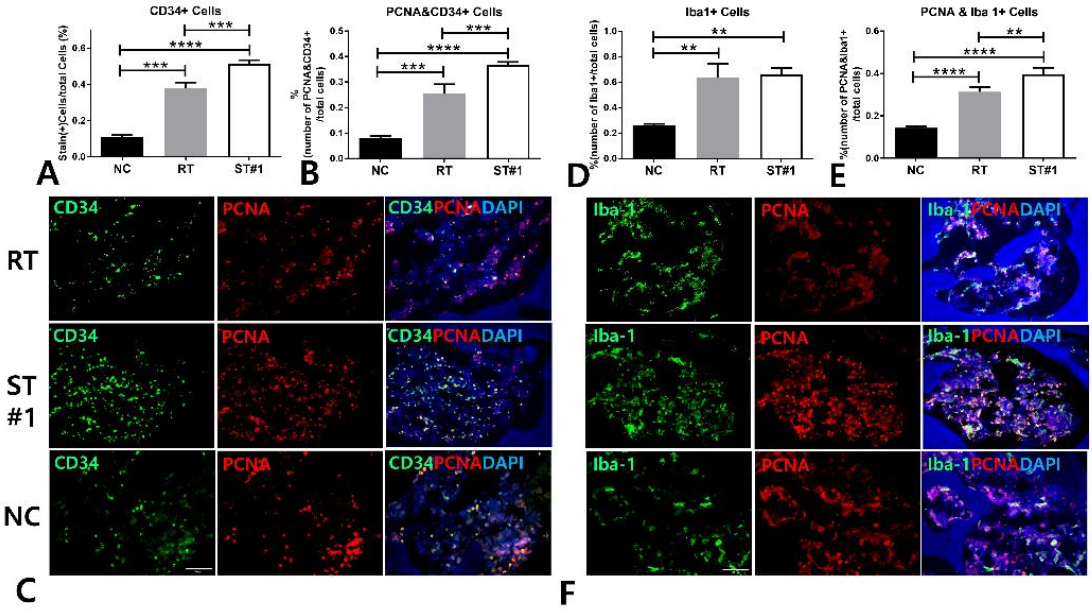
6. Figure 3: A scale bar should be indicated in the Photomicrographs.
   : A scale bar (50 μm) was inserted as shown in the figure below. (line 852)



7. Line 780: Check magnification (400x ?) in the legend to Fig.4.
   : Yes, the authors confirmed that it was shot at 400x. (line 865)

8. Figure 4: A scale bar should be indicated in the Photomicrographs.

   : Scale bar (50 μm) has been added to Figures C and F in Figure 4. (line 865)



9. Figure 5, panels E: IL-7R levels reported in the histograms are in contrast to what is described in the text (line 161)

   : Yes, thanks for the accurate point. We have edited the part you mentioned as follows. (line 418)

   "At the same time, IL-7R levels were significantly lower and CD45RA levels were significantly higher in the ST#1 group compared to those in the RT group."

10. Figure 5, panels G: CD45RA levels reported in the histograms are in contrast to what is described in the text (line 162)

    : Yes, thanks for the accurate point. We have edited what you said as follows. (line 418)

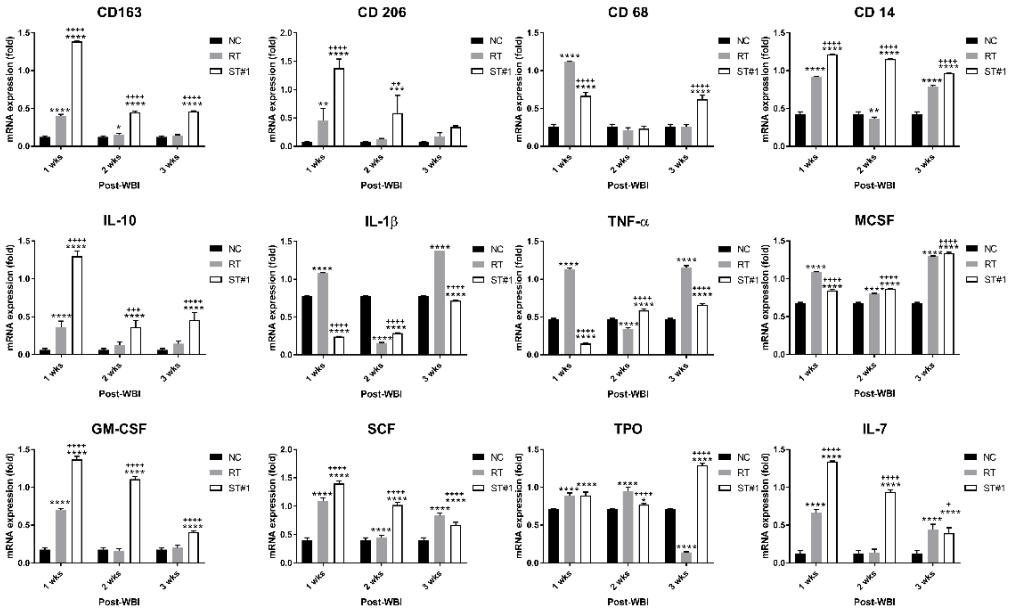
    "At the same time, IL-7R levels were significantly lower and CD45RA levels were significantly higher in the ST#1 group compared to those in the RT group."

11. Figure 5, panel J: A quantification of RT-PCR data would be suitable.

    : Yes, thanks to the good comments the authors have improved their papers. The RT-PCR band you mentioned was quantified and graphed. This has been added to supplementary figure 2 as follows. In addition, the text has been modified as follows. (line 426)

    "At the same time, IL-1β and TNF-α mRNA levels were lower in the ST#1 group than in the other groups during the first and second week post-WBI (Figure 5J, Supplementary figure 2). These results indicated that intraperitoneal ADSCs induced M2 polarization during repopulation post-WBI. The mRNA levels of GM-CSF, SCF, SDF-1, and IL-7 in the ST#1 group increased mainly in the first and second weeks post-WBI compared to those in the NC and RT

groups (Supplementary figure 2).”



12. Figure 6: Data on IL-2 levels are in contrast to what is reported in the text (line 187,188)
: Yes, thanks for the accurate point. We have edited what you said as follows. (line 442)
“Interestingly, G-CSF, C-X-C Motif Chemokine Ligand 13, and IL-2 levels increased in the ST2w group at week 2 post-WBI, which, with the exception of IL-2, was not observed in the ST3w group.”

Reviewer #2:

Specific Comments to Authors:

I would like to congratulate the authors for this manuscript. This study is interesting, resourceful and brings new perspective. I have some comments about the manuscript:

Materials and methods

1. Please check all the materials used for consistency /uniformity in writing (trademark, corporation name, city, state, country).
: Thanks for the good point The point you pointed out was corrected in the method and material part throughout this manuscript.

2. Line 380-383: please correct the sequence for chondrogenic and osteogenic staining accordingly with Alcian blue and Alizarin Red S.
: The part the reviewer said has been corrected as follows. (line 197)

"In the adipogenic, chondrogenic, and osteogenic groups, the culture medium was replaced every 2 d and the cells were stained with Oil Red O (Sigma-Aldrich), Alcian Blue (Sigma-Aldrich), and Alizarin Red S (Sigma-Aldrich), respectively, to confirm differentiation."

3. Line 372: Please explain how did you distribute P3-P7 cells into the treatment groups and clarify if there is any special appointment of certain passage into certain treatment group.
   : Thanks for the good point The authors looked at the experimental method of this paper once again. In this experiment, two kinds of ADSCs were used, mouse and human. While writing this paper, there was an error. Mouse ADSCs used in this experiment was P3~5, Human used P5~7. The related text has been modified as follows. (line 188)
   "Cell passage of mADSCs and human ADSCs (hADSC) were 3~5 and 5~7, respectively."

- Line 821: Please correct Table S1 into Table 1 as corresponds with Table 1 in line 441.
  : Thanks for the good point. Since there are many figures in this paper, Table S1 mentioned above has been unified as supplementary table 1 in both the text and legend. has been modified as follow. (line 252)
  "The sequences of human PCR primers for IL-4, IL-10, IL-1β, TNF-α, CD68, CD80, CD206, and β-actin as well as mouse PCR primers for IL-1β, TNF-α, CD14, CD68, CD163, CD206, IL-10, M-CSF, GM-CSF, SDF-1, TPO, IL-7, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), are listed in supplementary table 1."

Discussion

4. Please explain briefly about your reasons of using multiple passage cells(P3-P7).
   : Checking this experimental method once again, mADSC used P3~5 and hADSC used P5~P7. In this regard, the following has been added to the discussion. (line 580)
   "In the present study, mADSCs and hADSCs were used in the in vivo and in vitro experiments, respectively. In mice, P3~5 of mADSC were used, and P5~7 of hADSC were used; we recognize this to be a limitation in the interpretation of our results."

5. Please point out the limitations of your study within the methodology.
   : The same as previous answer.

Science editor

The manuscript entitled " Optimization of adipose tissue-derived mesenchymal stem cells transplantation for bone marrow repopulation after irradiation" is very interesting and would be valuable for the readers of the journal. However, significant revisions must be performed, in order the manuscript to be further processed.

1. In the beginning, regarding the used term mesenchymal stem cells that have been both used in the title and also in the text, in my opinion is not appropriate. Based on the latest nomenclature (Cytotherapy. 2019Oct;21(10):1019-1024. doi: 10.1016/j.jcyt.2019.08.002.) the MSCs are referred as mesenchymal stromal cells.
   : Yes, thanks for the good point. Adipose tissue-derived mesenchymal stem cells were changed to adipose tissue-derived mesenchymal stromal cells throughout this paper.

2. Also, the latest paper from IFATS (Cytotherapy 2013 Jun;15(6):641-8. doi: 10.1016/j.jcyt.2013.02.006.) refers to adipose derived stem cells, which are sharing specific properties and are characterized by different criteria compared to mesenchymal stromal cells. The authors need to clarify which cellular population are using for their experiments, accompanied by the verification experimental procedures (e.g. immunophenotyping, differentiation properties and morphology).
   : It is the same as the answer for question 8.

3. In the introduction section, the authors are not giving enough information, regarding the occurred health issue, but only referred to the acute irradiation syndrome. Should include information regarding the underlying disorders that probably can lead to bone marrow irradiation.
   : Thanks for the good point. As you suggested, the following related sentences have been added to the introduction of this paper. (line 93)
   "Hematopoietic symptoms, particularly those induced by ARS, develop into severe neutropenia and thrombocytopenia, which eventually lead to bleeding, infection, and death.[3] The same clinical results are obtained when myeloablative therapy is performed in patients with conditions such as leukemia, lymphoma, and myeloma.[4]"

   Also, the authors, should emphasize more on the regenerative properties and the ISCT or IFATs information for these cells.
   : The authors have added the following to the introduction to your request. (line 127)
   "ADSCs were first identified in 2001 by Zuk *et al.* as cells with multilineage differentiation characteristics.[25] ADSCs are characterized as a type of adult stem cells, i.e., pluripotent cells with limited differentiation capacity. The International Fat Applied Technology Society defines any fat-derived pluripotent cell population capable of adherent, proliferative, and differentiating capacity as an ADSC.[26] These ADSCs are able to differentiate into adipogenic, osteogenic, chondrogenic, myogenic, cardiac, and neuronal cells in vitro.[27–29] The plasticity and pluripotency of these ADSCs, as well as their clinically abundant and non-invasive harvestability, have enabled researchers to regenerate dead or damaged cells or organs.

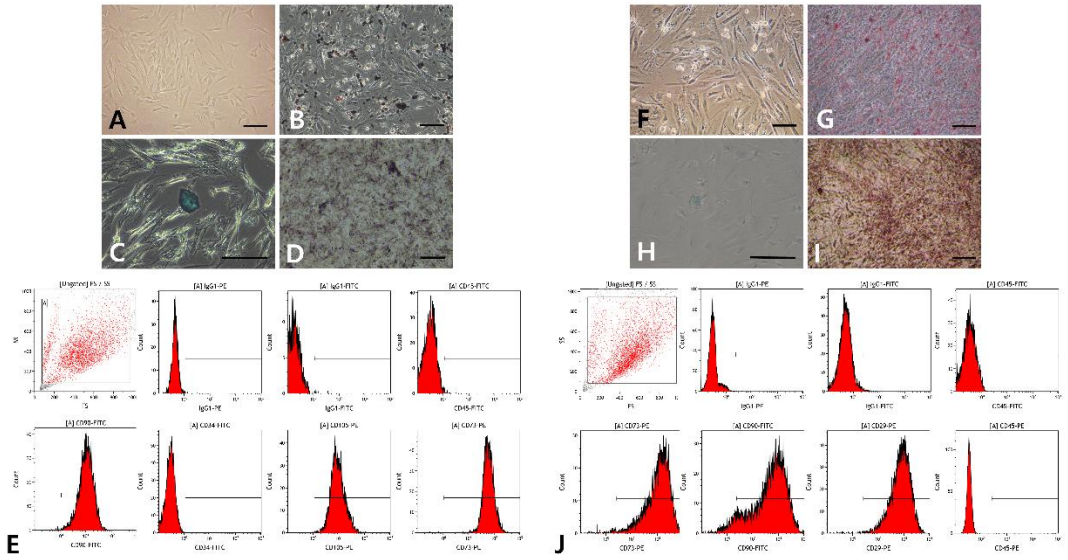
4. In addition, a major issue that i have noticed is that the authors did not follow WJSCs guidelines for the manuscript preparation. The authors should perform this change. The language suffers from grammar and phrase errors that should be corrected, e.g. the title must be change.
: In line with the guidelines of WJSC, the order of the paper have been changed. Additions include ORCID number, Author contributions, Core tips, Article Highlights, and more. The order has been moved so that the method section in the body goes to the front of results.

5. Another issue, is that the authors used MSCs derived from adult origin, that have limited proliferation capacity compared to the fetal MSCs. The authors need to add in their discussion, if it will be feasible enough to perform the same methodology in human subjects. What about using MSCs obtained from another sources such as umbilical cord or bone marrow.
: Thanks for the good comments. The content you requested has been added to the discussion section as follows.(line 573)
"Umbilical cord derived MSCs (UC-MSCs) from fetal tissue have better proliferation capacity than ADSCs, which are MSCs from adult tissue.[69] However, compared to ADSC, UC-MSC is difficult to collect and secure, and the culture success rate is low.[69,70] It is not yet known whether MSCs derived from various tissues will have the same effect on BM repopulation. However, according to previous studies, UC-MSC, BM-MSC, and ADSC showed similar immunomodulatory effects.[44,47,71] In this regard, future research should evaluate variation in treatment efficacy according to the origin, age, and passage of MSCs. In treating BM repopulation with MSCs in humans, future clinical trials should specifically consider the dose to be administered."

6. Regarding the experimental procedure, i am not sure if the statistical analysis has been appropriately performed. The authors need to give more information regarding their data with supplementary material.
: Thanks for the good comments. The statistical processing method for the data of this study was checked again and the method section was modified as follows. (line 348)
"The Kaplan–Meier method was used to estimate the distribution of survival rates over time. A repeated measures two-way repeated measures ANOVA with Sidak's or Tukey's multiple comparison test was used to compare groups over time. A Kruskal–Wallis test and Dunn's multiple comparison test were used to compare the three mouse and human experimental groups, respectively. The GraphPad Prism 7 software for windows (San Diego, CA, USA) was used for statistical analysis."

7. In the materials and methods and results sections, i did not find anywhere the characterization of the used MSCs of the current study. The authors should follow the criteria outlined by ISCT or IFATs, in order to define their cell population.
: Thanks for the nice comment. The part you mentioned was composed as a supplementary

figure 1 and added as follows. It is about the surface markers and multilineage differentiation assay for mouse ADSCs and human ADSCs used in this experiment



: Methods related to this have already been described in the first paragraph of Multilineage differentiation assay in line 196 and Flow cytometry in line 275. However, in the results section, the description is missing, so we have added the following. (line 357)

"**Characterization of mouse and human ADSCs**

Mouse and human ADSCs appeared as flat cubic cells in initial culture, and changed to spindle-shaped cells similar to fibroblasts with successive subcultures. (Supplementary figure 2A, F). ADSCs were differentiated into adipose, cartilage, and bone, as evidenced by staining with Oil Red O and toluidine blue, von Kossa, respectively. (Supplementary figures 2B-D, G-I). Surface markers of cultured human ADSCs, which were passaged 3–5 times, were over 95% positive for CD73 and CD105, but negative for CD45 and CD34 (Supplementary figure 2E). In the case of mouse ADSCs, CD29, CD73, and CD90 were positive, and CD34, CD45, and CD31 were negative. (Supplementary figure 2J)"